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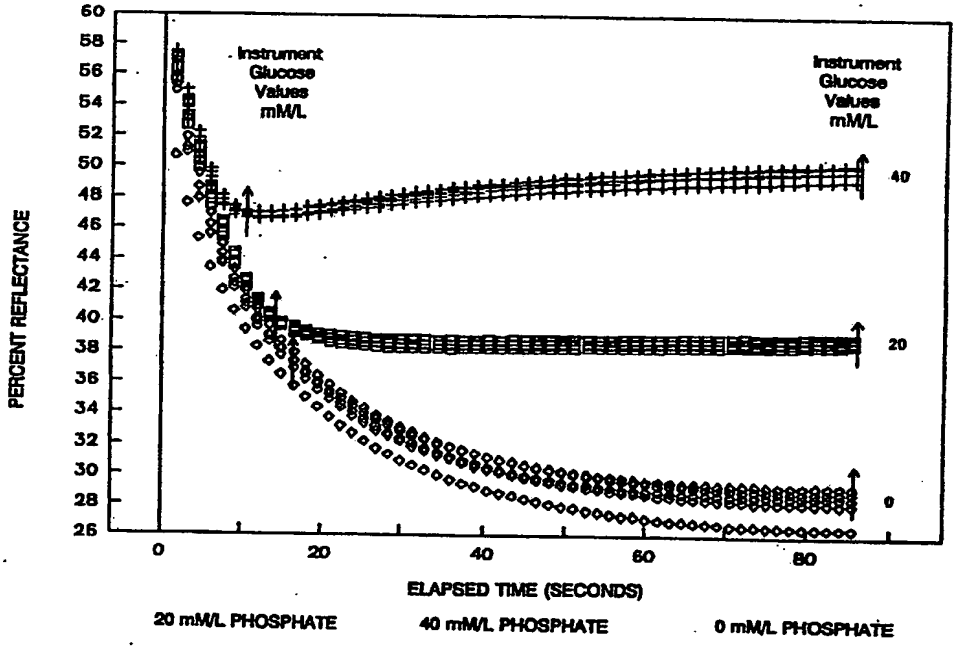
(54) Title: LIQUID GLUCOSE CONTROL SOLUTION AND PROCESS OF MAKING THE SAME



(57) Abstract

A liquid glucose control solution includes water, a predetermined amount of glucose, phosphate as a reaction rate regulator and xanthan as a soluble gel. An alternative control solution may additionally include fixed red blood cells. Other materials such as a disinfectant may be added. The invention furthermore contemplates a process of making the liquid glucose control solution by mixing the required components together.

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Liquid Glucose Control Solution and Process of Making the Same

Background of the Invention

The present invention is directed generally to a glucose control solution for use in establishing the validity of dry reagent glucose test strips, and more particularly to a liquid glucose control suitable for use in glucose analyzer systems that give quantitative measures of glucose in blood or serum. The control solution includes water, a reaction-rate regulator, a gel which does not change viscosity with temperature, and glucose.

The level of glucose in the blood is determined by the amount of carbohydrate ingested and by available insulin. The diabetic may produce either excess insulin and have an abnormally low blood glucose level or, insufficient insulin which will result in an abnormally high level of blood glucose. Both circumstances lead to serious health problems for the diabetic. Monitoring the level of glucose in the blood is therefore important to the management of diabetes.

Dry reagent strips are widely used for detecting glucose in urine and blood. In general, such test strips comprise plastic strips provided at one end thereof with an absorbent paper portion impregnated with a detector system, e.g., an enzyme system and a color indicator compound which changes color when oxidized. The detector system is normally covered with a porous membrane filter. The change in

color can be measured by comparing the strip to a color chart calibrated to various glucose concentrations. More recently, however, to enable the blood glucose level to be more accurately controlled, instruments have been developed to measure the color change in a reflectance photometer and thereby give quantitative results. One such instrument is commercially available from Diagnostic Laboratory Systems Division, Boehringer Mannheim Corporation and is marketed under the name Accu-Chek®Easy™. This instrument is designed for use by diabetics so that they can monitor their blood glucose level.

Those skilled in the art know that controls or standards are required for these glucose analysis dry chemistry systems to assume that correct measurements are obtained. This need was met for most current systems with a stable blood glucose control which was the subject of U.S. Patent No. 4,729,959. Diabetics who self-test for blood glucose with the use of dry chemistry systems have concerns with the use of controls containing red blood cells. Prior art aqueous solutions of glucose do not give acceptable results in the Accu-Chek®Easy™ instrument.

Accordingly, a primary object of the invention is to provide an improved liquid glucose control solution.

Another object of the invention to provide a control solution with reaction kinetics providing rapid accurate readings stable over ninety seconds when used with a dry reagent strip.

Another object of the invention is to provide a control solution that produces reproducible readings

within a normal range of temperatures.

Summary of the Invention

The liquid glucose control solution of the present invention includes a stable liquid control solution including water, a predetermined amount of glucose, phosphate as an anionic component, and xanthan as a soluble gel. An alternative control solution may additionally include fixed red blood cells. Other materials such as a disinfectant may be mixed with the above four required components. Another aspect of the invention is a process of making the liquid glucose control solution by mixing the four required components together.

Brief Description of the Drawings

Figure 1 is a graph showing the effect of different phosphate concentrations on the reaction kinetics resulting from use of the present invention.

Description of the Preferred Embodiments

Whereas the liquid glucose control solution of the present invention is designed to operate with an instrument called the Accu-Chek®Easy™, it will also operate with many other instruments. The objectives of the control are to provide reaction kinetics comparable to blood; to defuse or chromatograph like blood, and to give responses that are not temperature dependent.

Any convenient method can be used to formulate the glucose reference control of the invention. One preferred procedure involves first

making up an aqueous glucose solution by adding glucose to distilled water followed by the addition of the essential phosphate and xanthan components and any optimal components such as a disinfectant. Fixed red cells may also be added to the control as in Ryan, U.S. Patent No. 4,729,959.

The water is used to create a reagent solution. With respect to the glucose, "predetermined" means that, prior to formulation of the actual reagent, a concentration of glucose has been selected. This concentration may vary as will be recognized by those skilled in the art. Ryan U.S. Patent No. 4,729,959 discloses a range of from 2.22 mM/L to 27.8 mM/L, but lower ranges to about 1.11 mM/L are possible. A typical range would be from about 3.33 mM/L to 16.7 mM/L. The units expressed are millimolar per liter.

Kinetics

Phosphate is included as a reaction rate regulator. It is desirable for the reaction to proceed rapidly and then plateau to a constant value. This pattern produces a result that is less time dependent. Thus, the meter can be read for example in 10 seconds or 60 seconds and the result will be the same.

Figure 1 shows the reflectance values obtained at different times and the effect of phosphate ions on the process. As the phosphate is increased, lower initial reflectance values (more color) are attained. However, when 40 mM/LPO_4 is used, there is a decoloration that occurs with time.

The ideal concentration is the 20 mM/LPO_4 where the reflectance is constant with time. The sample with no PO_4 produces such a slow reaction, that equilibrium is not reached until 70 seconds have passed. Accordingly, a phosphate concentration of about 5 to 35 mM/L may be used with about 10 to 30 being more preferred and about 20 mM/L being most preferred. These concentrations of phosphate are best for glucose concentrations of 2.8 mM/L - 13.9 mM/L. At higher concentrations of glucose, more phosphate is required and less at lower concentrations.

Other anions such as citrate or EDTA operate to produce rapid kinetics but are inferior to phosphate because they decolorize over time somewhat like the plot of the 40 mM/L concentration of phosphate in Figure 1.

Diffusion or Chromatography

To obtain an appropriate test, the glucose containing solution must move to the analysis system in a similar manner as blood. This requires that a thickening agent or gel be used. These agents include polyvinylpyrrolidone, polyvinyl alcohol, polyethylene glycol, dextran and bovine serum albumin. None of these agents provide any special characteristics except to make the solution viscous. In Kennamer, U.S. Patent No. 5,028,542, polystyrene sulphonate is claimed as a viscosity agent. As in the above, this agent simply increases viscosity. The gel that is used in the present invention is xanthan, a polysaccharide. This provides special characteristics to the product. Xanthan, unlike other gels, does not change viscosity with temperature. The viscosity of

the solution containing xanthan gum experiences almost no change with temperatures up to 93°C (200°F). A change in viscosity produces erratic values and variation in results with temperature. In addition, this gel produces uniform chromatography or diffusion of the control. This provides greater precision as shown by the data of the following Table I.

TABLE I

Effect of xanthan gum has on the chromatography and precision of the Accu-Chek®Easy™

1.) Sample #1 (w/xanthan)

15.6 mM/L	Na_2HPO_4
4.7 mM/L	NaH_2PO_4
.1g/L	Proclin 300
3.9g/L	Xanthan Gum
8.33 mM/L	Glucose

Accu-Chek®Easy™ Recovery

S.D.	C.V.
2.31	0.91

2.) Sample #2 (w/o xanthan)

15.6 mM/L	Na_2HPO_4
4.7 mM/L	NaH_2PO_4
.1g/L	Proclin 300
8.33 mM/L	Glucose

Accu-Chek®Easy™Recovery

S.D.	C.V.
21.63	10.44

Table I shows that both the standard deviation and coefficient of variation were about tenfold less in the series of tests with xanthan as

compared to the series of tests using the control solution without xanthan. Xanthan may be used in concentrations of about 1 to 5 g/l, more preferably 2 to 4 g/l and optimally about 3 g/l.

Example I

A control for the Accu-Chek®Easy™ is prepared by dissolving per liter

15.6 mM Na_2HPO_4
4.7 mM NaH_2PO_4
3.00 gm xanthan

To this preparation is added the desired amount of glucose. After mixing to dissolve the components and gel, an appropriate disinfectant is added.

Example II

A control for the Accu-Chek®Easy™ is prepared by dissolving per liter:

15.6 mM Na_2HPO_4
4.7 mM NaH_2PO_4
3.00 gm xanthan
$0.1x12^{12}$/dL fixed bovine red cells

To this preparation is added the desired amount of glucose. After mixing to dissolve the components and gel, an appropriate disinfectant is added.

Example II describes the addition of fixed red blood cells. Fixed red blood cells can be

obtained by using conventional red blood cell fixing agents known in the art as, for example, aldehydes such as formaldehyde and glutaraldehyde, and imidinating agent such as dimethylsuberimidate or other chemical fixative agents. Any animal red blood cells can be utilized, but human and bovine red blood cells are preferred.

Fixing of the red blood cells is readily accomplished by treating a suspension of the red blood cells with a sufficient concentration of the fixing agent. The amount of fixing agent added to the suspension of red blood cells will vary depending upon the number of cells in suspension being treated and the fixing agent employed. In the case of aldehyde and imidinating fixing agents, the concentration will usually vary from 0.004 to 1.0% by weight per $0.1x10^{12}$/dL of red blood cells. A preferred concentration of red blood cells is about 0.1 to $0.3x10^{12}$/dL. In all cases, the reaction of the fixing agent with the red blood cells is allowed to proceed until their ability to metabolize glucose is completely inhibited. The fixing period necessary to achieve this result ordinarily takes about 24 to 48 hours.

Figure 1 shows the reflectance values obtained at different time points and the effect of phosphate ions on the process at a constant glucose concentration. As the phosphate is increased, equilibrium is attained much quicker, resulting in higher reflectance values (less color).

I claim:

1. A liquid glucose control solution for use in establishing the validity of dry reagent glucose test strips for indicating amounts of glucose in blood, said solution comprising,

water;

a predetermined amount of glucose;

phosphate as a reaction rate regulator in a concentration of about 5 to 35 mM; and

xanthan.

2. The liquid glucose control solution of claim 1 wherein the concentration of xanthan is about 2 to 4 g/1.

3. The liquid glucose control solution of claim 1 wherein the concentration of xanthan is about 3 g/1.

4. The liquid glucose control solution of claim 1 wherein the concentration of phosphate is about 10 to 30 mM/L.

5. The liquid glucose control solution of claim 1 wherein the concentration of phosphate is about 20 mM/L.

6. The liquid glucose control solution of claim 4 wherein said phosphate comprises sodium phosphate.

7. The liquid glucose control solution of claim 2 wherein said predetermined amount of glucose comprises about 2.22 to 27.8 mM/L of glucose.

8. The liquid glucose control solution of claim 1 further comprising about 0.1 to $0.3x10^{12}$/dL red blood cells fixed with a fixing agent to render said red blood cells incapable of metabolizing glucose, the number of said fixed red blood cells being sufficient to provide a glucose reference control for glucose test strips in which the true value of glucose and the measured value is approximately the same.

9. The liquid glucose control solution of claim 8 wherein the red blood cell concentration is about 0.2 to $0.3x10^{12}$/dL.

10. The liquid glucose control solution of claim 8 wherein the red blood cell concentration is about $0.3x10^{12}$/dL.

11. The liquid glucose control solution of claim 8 wherein the red blood cells are human red blood cells.

12. The liquid glucose control solution of claim 8 wherein the red blood cells are bovine red blood cells.

13. A process for making a liquid glucose control solution for use in establishing the validity of dry reagent glucose test strips, comprising mixing water, a predetermined amount of glucose, phosphate in a concentration of about 5 to 35 mM/L, and xanthan in a concentration of about 1 to 5 g/1.

14. The process of claim 13 further comprising mixing said xanthan in a concentration of about 2 to 4 g/1.

15. The process of claim 13 further comprising mixing said xanthan in a concentration of about 3 g/1.

16. The process of claim 13 further comprising mixing said phosphate xanthan in a concentration of about 10 to 30 mM/L.

17. The process of claim 16 further comprising mixing said phosphate in a concentration of about 20 mM/L.

18. The process of claim 13 further comprising mixing about 0.1 to $0.3x10^{12}$dL red blood cells fixed with a fixing agent to render said red blood cells incapable of metabolizing glucose, the number of said fixed red blood cells being sufficient to provide a glucose reference control for glucose test strips in which the true value of glucose and the measured value is approximately the same.

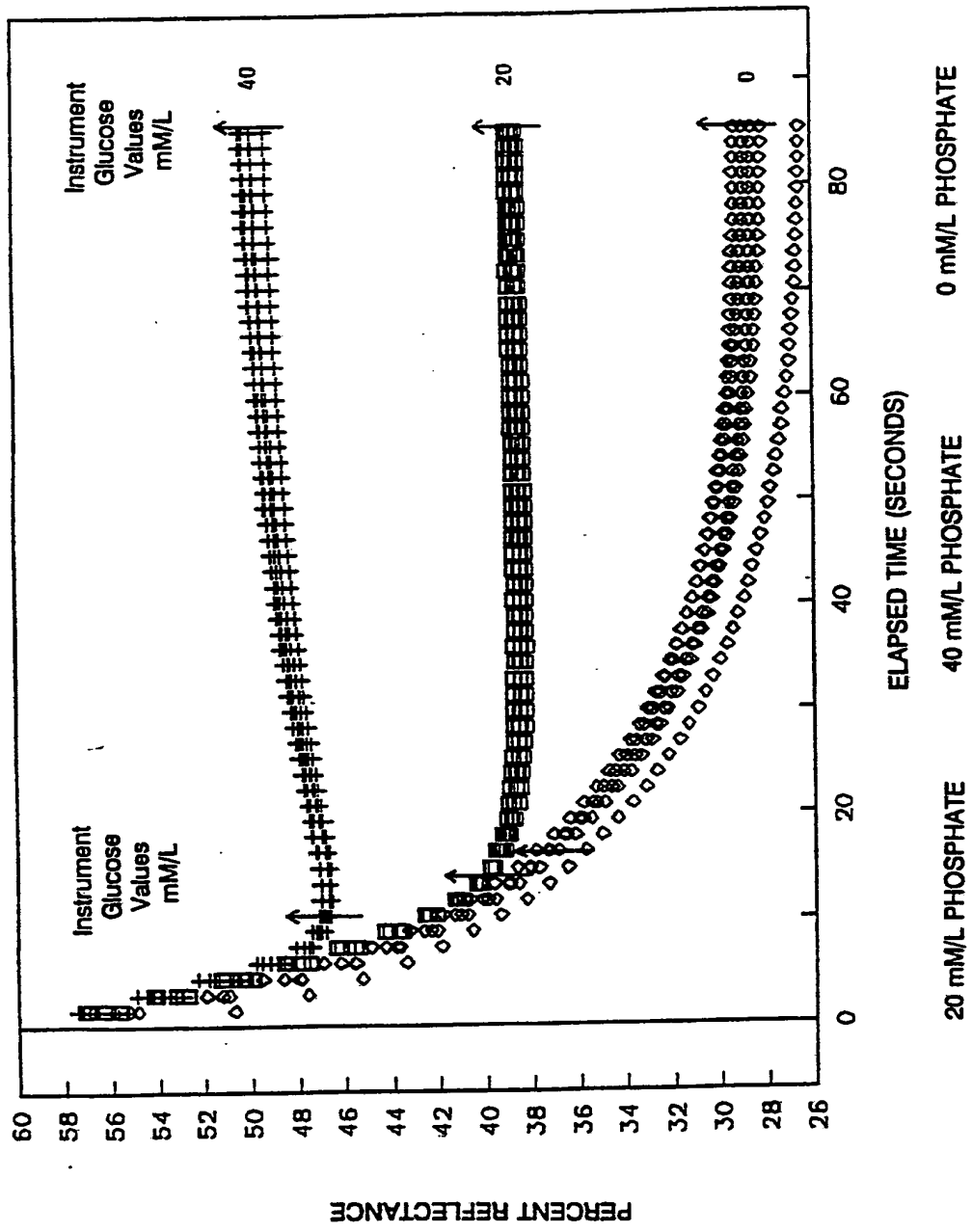
Instrument Glucose Values mM/L
Instrument Glucose Values mM/L
PERCENT REFLECTANCE
ELAPSED TIME (SECONDS)
20 mM/L PHOSPHATE
40 mM/L PHOSPHATE
0 mM/L PHOSPHATE

FIG.1

INTERNATIONAL SEARCH REPORT

In. .ational application No.
PCT/US93/03799

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 31/70; G01N 31/00; A01N 1/02
US CL :514/23, 54; 435/14; 252/408.1; 424/601

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/23, 54; 435/14; 252/408.1; 424/601

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS Online: search terms: glucose, xanthan, phosphate# and control or standard or reagent

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,729,959 (RYAN) 08 March 1988, see entire document.	1-18
Y	US, A, 3,920,580 (MAST) 18 November 1975, see entire document.	1-18
Y	US, A, 4,572,899 (WALKER ET AL) 25 February 1986, see entire document.	1-18
Y	US, A, 5,028,542 (KENNAMER ET AL) 02 July 1991, see entire document.	1-18
Y	G.O.PHILLIPS et al, "GUMS AND STABILISERS FOR THE FOOD INDUSTRY 4," Published 1987 by IRL Press (Wash.D.C.), see especially pages 363-369.	1-18

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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Date of the actual completion of the international search
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International application No.
PCT/US93/03799

C (Continuation).. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
Y	Chemical Abstracts, Vol. 113, issued 1990, Harada et al, "Electron Microscopic Studies on Molecular Association in Gels of Curdlan and Other Polysaccharides in Food." see page 592, abstract no. 189906r, Kobe Joshi Daigaku Kiyo, 23(2):137-153.	1-18

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